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Poster session: Genetic alterati ns, tumor suppressor genes, prognostic factors

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IDENTIFICATION OF GENES ASSOCIATED WITH PROSTATE CANCER DEVELOPMENT.

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Identification of genes specifically expressed in tumor cells but not in normal cells, or *vice versa*, is important for the understanding of the molecular basis of carcinogenesis. Furthermore, such genes may provide us with markers for early tumor detection. Recently, the technique of differential display has been proven to be a powerful tool to identify and clone differentially expressed genes. The technique involves the reverse transcription of messenger RNA using an anchored oligo-dT primer, followed by a PCR amplification reaction using several different, arbitrarily chosen primers and subsequent seperation of the products on a denaturing gel. In the study presented here, messenger RNA from normal, benign hyperplastic and tumor prostatic tissue from the same patients was extracted and used for differential display analysis. Twenty different combinations of primers were tested. Twelve apperently differentially expressed mRNAs (overexpressed in tumor or in normal tissue) were identified this way. The complementary DNA fragments were recovered from gel, reamplified and used as probes for Northern analysis. One of the probes (DD3) detected two transcripts (2.2 and 4.0 kb) that are specifically expressed in human prostatic tumors (9 out of 12 tumors studied) whereas no expression of these transcripts was found in normal or benign hyperplastic prostate tissue. Nucleotide sequence analysis showed no homology to any known gene. Currently, more sequence data are being obtained as well as studies are being performed to analyse the tissue-specificity of DD3 expression and expression in other tumors. In conclusion, differential display is a useful approach to identify differentially expressed genes in prostate tumor development and DD3 might be useful in prostate cancer detection.

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GENETIC ALTERATIONS IN LOCALIZED PROSTATE CANCER: IDENTIFICATION A COMMON REGION OF DELETION ON CHROMOSOME 16q.

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Accumulation of mutations in oncogenes and tumor suppressor genes transforms a normal cell into a malignant cell by allowing it to escape from normal control of growth. For prostate tumorigenesis, the current model defines specific mutations of the TP53 tumor suppressor gene and loss of heterozygosity (LOH) for loci on chromosomes 8p, 10q, 16q and 18q. In order to determine if alterations frequently found in other adenocarcinomas (breast, ovarian, gastric, colorectal) including losses of heterozygosity on chromosomes 1p, 3p, 7q, 11p, 17p, 17q and 18q, and amplification of c-myc, c-erbB-2/neu oncogenes and the 11q13 region are also involved in prostate cancer, we examined 21 localized early stage prostate tumors. We detected no amplification of the c-myc, c-erbB-2/neu proto-oncogenes and 11q13 region (int2/FGF3), or mutations of the TP53 gene. Allelic losses were found in chromosomal regions 10q (20%), 7q (33%) and 16q (33%). Furthermore, as the first step toward isolation of tumor suppressor genes on 16q, we used six polymorphic markers and identified a common region of deletion between the 16q centromere and the D16S19 locus.

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DELETION MAPPING OF CHROMOSOME 18q IN PROSTATE CANCER (PC) AND TRANSITIONAL CELL CARCINOMA OF THE BLADDER (TCC).

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Somatic allelic loss is a hallmark of tumour suppressor gene (TSG) inactivation. To investigate the potential role of the TSG DCC [deleted in colorectal carcinoma] in two common urological cancers, 30 PCs and 31 TCCs were allelotyped at 5 chromosome 18q loci using polymorphic DNA probes: pL2.7 (18q12); pOLVIIA8 (18q12.1-21); p15-65, SAM 1.1, JOSH 4.4, DCC 1.9 (all within DCC, 18q21.3) - B.Vogelstein, Baltimore; pOS-4 (18q22) and pL159-1 (18q23).

7/26 (27%) of PCs and 8/25 (32%) of TCCs exhibited loss of heterozygosity (LOH) on 18q, including DCC in 6 PCs and all 8 TCCs. LOH correlated with advanced stage in PC and with TCC recurrence or muscle-invasion (both $p<0.05$). In one PC and 5 TCCs, centromeric markers also exhibited LOH; one PC and 2 TCCs retained centromeric alleles. 4 PCs and 7 TCCs also exhibited LOH at telomeric loci. One PC exhibited LOH only telomeric to DCC. No TCC retaining heterozygosity at DCC exhibited LOH elsewhere. The regions of commonest deletion (RCD) were 18q22-23 in PC and 18q21.3-23 in TCC.

These results suggest the presence of a late-acting TSG on 18q in both cancers. DCC is implicated as the target TSG in TCC, located within the RCD; that for PC may be telomeric to DCC. (Supported by a SWRHA research grant).

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ANGIOGENIC ACTIVITY OF TWO HUMAN PROSTATE TRANSPLANTABLE CELL LINES.

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Angiogenesis is the generation of new capillary blood vessels which occurs by a process involving the proliferation, migration and maturation of endothelial cells, a prerequisite for tumour growth and metastases. In 1985 a transplantable cell line (Ten12) was established in nude mice from a primary human prostatic cancer. The particular feature of this patient's tumour was its high degree of vascularisation, a characteristic that has been maintained in the mouse xenograft, presumably due to the release of one or more angiogenic factors.

Initial studies using serum-free conditioned medium from primary *in vitro* cultures of Ten12 on logarithmic cultures of bovine aortic endothelial (BAE) cells did not show a significant increase in the population doubling time. Morphological transformation of BAE cells however was observed, from the normal cobblestone growth to cells exhibiting a more elongated shape and with the appearance of sprouting cells self-associating into a complex network of tube-like structures. Serum-free conditioned medium from LNCaP, an established human prostate cancer cell line, did not affect the morphology of BAE cells to the same extent.

The effect of primary *in vitro* cultures of Ten12 were compared with those of Ten15 (another transplantable human prostatic cancer cell line established from a metastatic lesion which exhibits a lower degree of vascularisation) on both logarithmic and quiescent BAE cells using an insert co-culture system. Growth curves for both lines were consistently higher than controls and those observed with conditioned medium, but were not statistically significant. Greater morphological changes to BAE cells were however observed with co-cultures of Ten12 cells than those shown with Ten15 cells.

Angiogenic effects on macrovascular and microvascular endothelial cells are reported to differ. We therefore recently assessed the effects of conditioned medium and primary *in vitro* co-cultures of Ten12 and Ten15 cells on microvascular endothelial cells derived from bovine adrenal capillaries (BAC). Initial identification of which angiogenic factors are important for the vascularisation of Ten12 tumours will be attempted using a panel of neutralising antibodies and electrophoretic analysis.